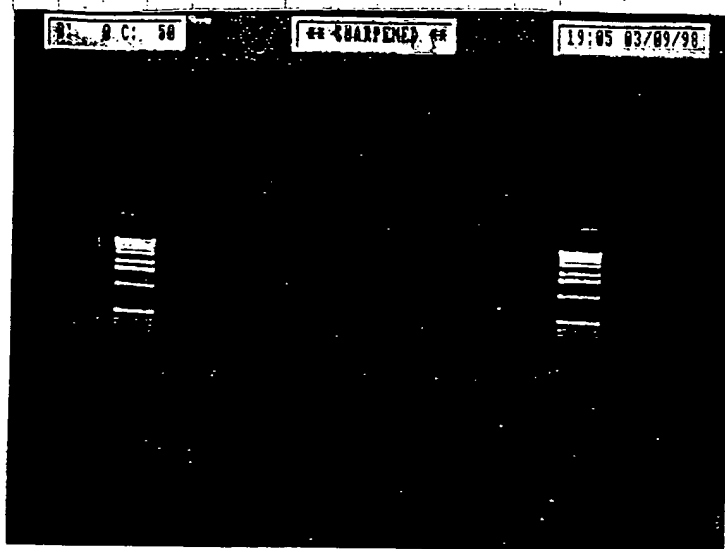
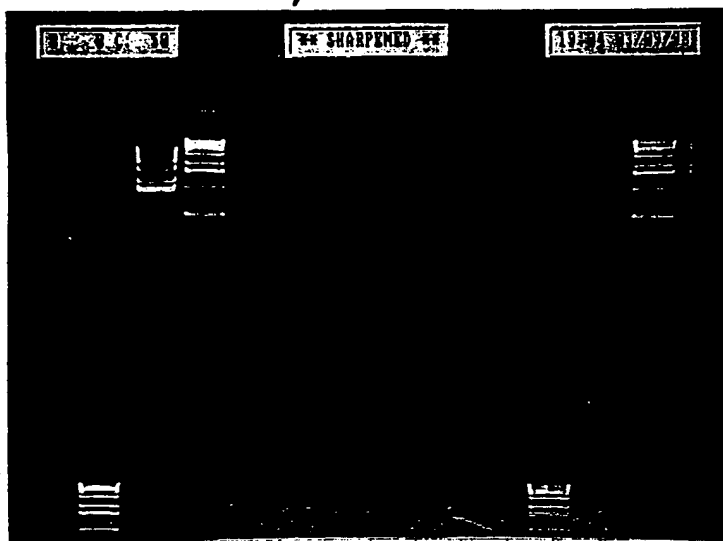


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49665 200bp



sourcing for 49665

18  
 1> 100ng ssb  
 90 5> plentag buffer  
 18 1> dNTP  
 18 1> forward  
 18 1> reverse  
 18 1> plentag  
 720 40> old H<sub>2</sub>O  
 50> total

95°C 5min 1 cycle

95 1min  
 57 1min  
 72 1.5min

20 cycles

1.5% TAE gel

228 may possibly have the band

Thoughts try DMSO w/ amplitag and see if this helps

To Page No. \_\_\_\_\_

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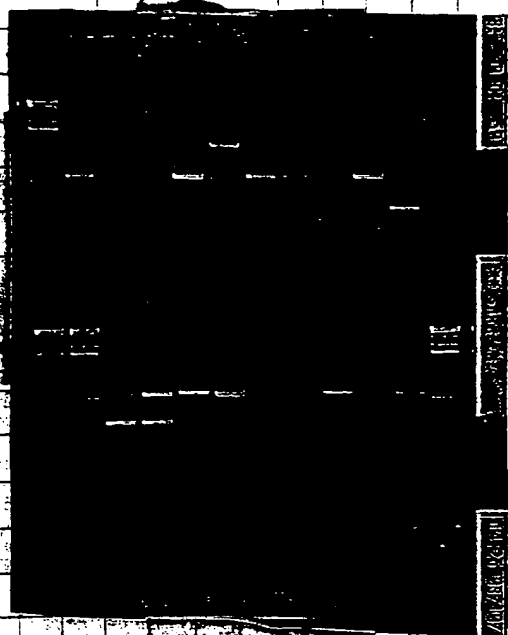
Invented by

Bethanne Dowl

Date

Recorded by

From Page No. \_\_\_\_\_



PCR sourcing w/ 5% DMSO & tag

	20
1x ss lib	
5x buffer	1100
3x MgCl <sub>2</sub>	600 600
2.5x DMSO	55
1x forward	220
1x reverse	220
1x dNTP	220
1x amplitag	220
to 50x ddH <sub>2</sub> O	759

95°C 5 min 1 cycle

95° 1 min

56° 1 min

72° 1 min 25 cycles

product should be around 200bp

277 278 294 possibilities

run gel and do Southern  
Order

278 from yesterday	229D
26	229
94	230
99	247
13.5	247
153	255
154	293
227D	294
227	301
228	302

~~228D~~  
~~228~~

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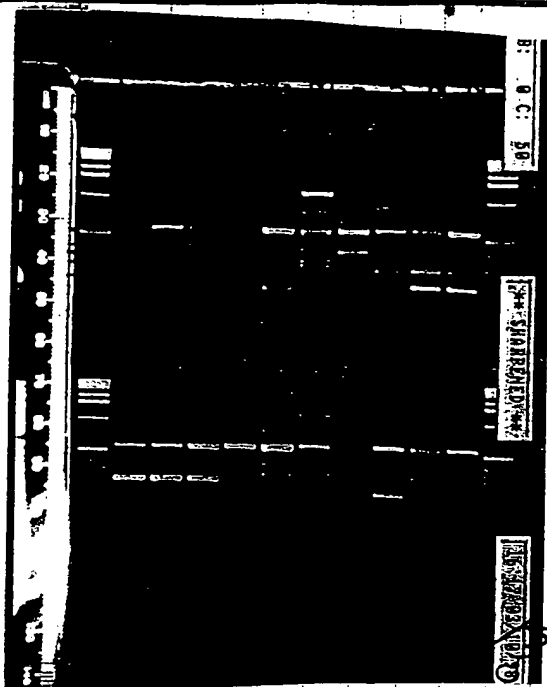
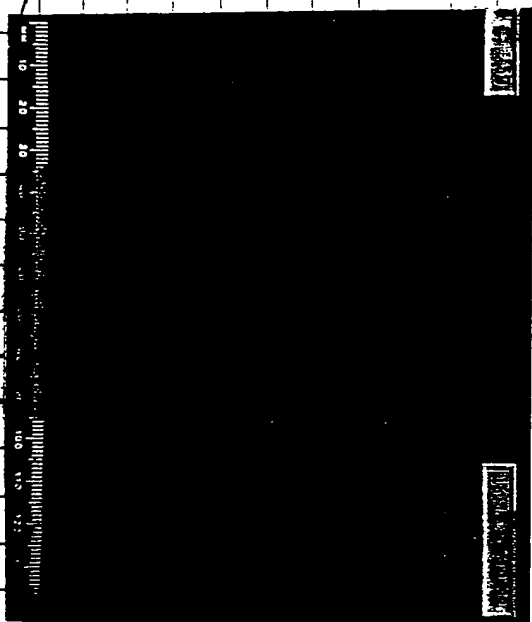
Invented by

Bethanne Zemel

Date

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From Page No. —



1st amps add 500x transfection + 500x compl  
let rock like add 10 ml media

1 56436  
5 57695  
9 56350  
15 57694  
17 57834  
4 58800

let go until Friday  
then harvest cells.

Althoughts Southern for 49665 cytokine.  
Probably 227 or 228 has it  
If not try 254 etc

Take apart southern. Neutralize and bake  
2-3 hrs in 80° vacuum oven

To Page No. —

Witnessed &amp; Understood by me,

Date

Invented by

Bithanne Devel

Date

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PAGE: 1

USER: 5 ID: 32P MAXI

PRESET TIME: 1.00

PRINTER: STD

RS232: OFF

H#: YES

SCR: NO

RCM: YES

COMMENTS:

SAMPLE REPEATS: 1

REPLICATES: 1

MULTIPLIER: 1.000000

DATA CALC: CPM

COUNT BLANK: NO

VIAL SIZE: MAXI

ISOTOPE 1: 32P %ERROR: 0.00 BKG. SUB: 0 HALF LIFE: YES

SAM POS  
NOTIME  
MIN

H#

32P  
CPM %ERROR

RCM

ELAPSED  
TIME

SAM NO	POS	TIME MIN	H#	CPM	%ERROR	RCM	ELAPSED TIME
1	1-1	0.51	49.0	86389.21	0.95	0.00	1.38

make probe 49665

1x 49665 pl

10x 8<sup>32</sup>P ATP

6x T4 pnt buffer

2x T4 pnt

41x ddH<sub>2</sub>O

mix and incubate 30 min 37°C

clean

dilute 1:10 and count

Miniprep + and do Xba digests

5x cDNA

2x buffer HNM402

1x Xba

12x ddH<sub>2</sub>O

32

104

32

384

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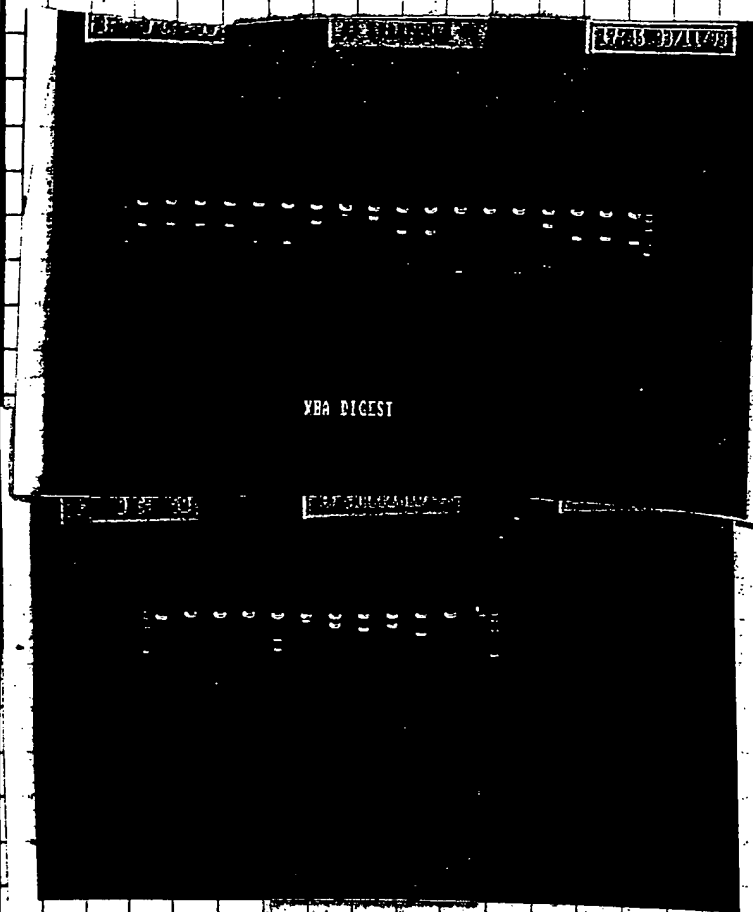
Invented by

B. Thorne Devel

Date

Record d by

From Page N



52159

(3)

(4)

(5)

(7)

any

52721

3

(4)

(5)

52165

2 (M)

(3)

52163

(2)

(3)

any

52175

(1)

(3)

(4)

any

52035

1

?

(2)

(4)

(5)

any

52161

1

(2)

(7)

52777

(1)

(5)

?

replate peno

52765

(1)

(2)

(3)

52162

(1)

?

replate

52160

1

42836

2

→ ?

To Page No.

Witnessed &amp; Understood by me,

Date

Inv nt d by

Bethanne Daniel

R c rded by

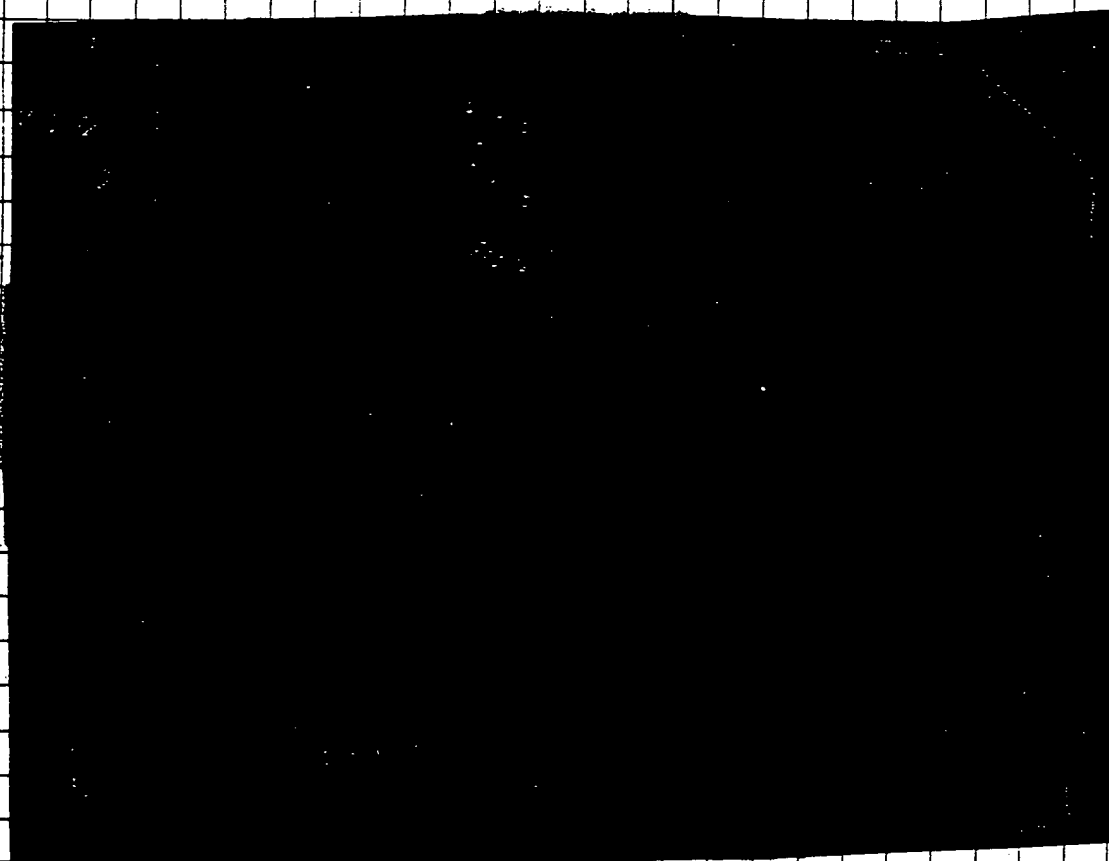
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Wash Southern

2XSSC + 0.1% SDS	42°	40 min
0.5XSSC + 0.1% SDS	42°	15 min
0.1XSSC + 0.1% SDS	42°	10 min

expose to film 2 hrs develop

Source for cloning 49665  
227, 228, 294

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In ss d &amp; Understood by me,

Date

Invented by

Bethanne Deuel

Recorded by

Dr. A.

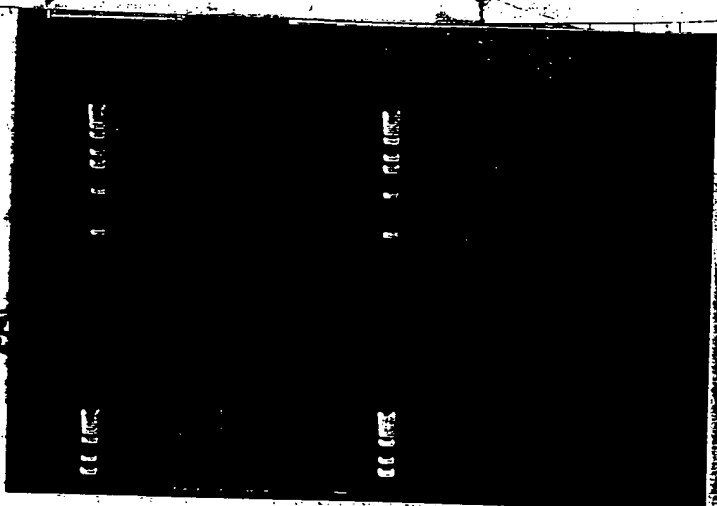
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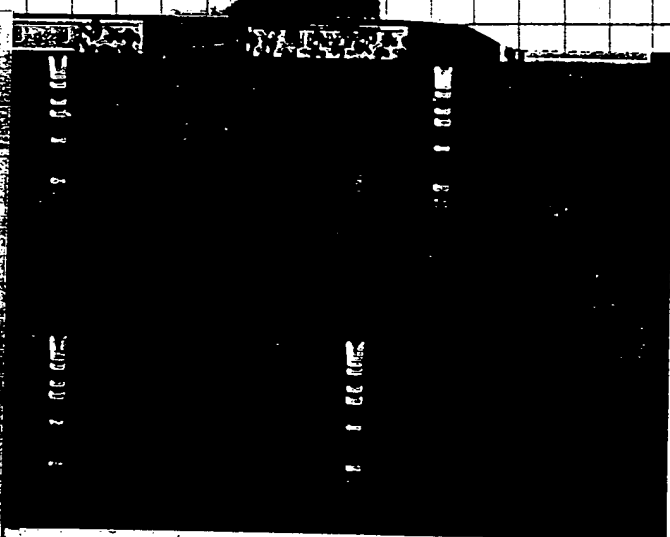
154? 247? 255?

38237

1036



38085 - 28067 4620



60684 ~150

Cloning

~ 49665 - wash buffers

2XSSC + 0.1% SDS 40 min

0.5XSSC + 0.1% SDS 20 min

late air dry expose to film develop

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R c rded by \_\_\_\_\_

Date \_\_\_\_\_

Sourcing  
 run PCR to check size of  
 60684  
 the bottom bands  
 are 150

so use 26, 153, 254, or 247  
 for cloning

Cloning - 1 pick + for 49665  
 grow O/N  
 $39 + \frac{26}{65} +$  in 17,000 colonies

2) Transform and plate rescues from yesterday

PCR for new sourcing

60748 - 160 lib 56° annealing

38085 - 8 lib 12 diff annealing temp

95° 5 min 1

95° 1 min

52-63° 1 min

72° 1 min 10 sec 25

72°C 5 min 1

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With ss d &amp; Understood by me,

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do miniprep on 49665

Baculovirus

set up 1st amp

Cloning

make probes for

52781	36456	36967	1097A	0.5 $\mu$ probe	12
601684	38096			3 $\mu$ pnc buffer	36
60748	36461			5 $\mu$ $\gamma$ - <sup>32</sup> PATP	60
38085	35735			2 $\mu$ T4 pnc	24
38237	36963			19.5 $\mu$ ddH <sub>2</sub> O	234

clean probes in Purol quant G<sup>50</sup> micro columns  
freeze

set up PCR for sourcing of 14 oligos in  
12 libraries

plate virus from yesterday

set up PCR for sourcing of 49665

1 $\mu$ bugs	12		
5 $\mu$ $\alpha$ lantaq buffer	60	—	
1 $\mu$ forward 49665	12	—	
1 $\mu$ reverse 49665	12	—	
1 $\mu$ dNTP	12	—	
1 $\mu$ $\alpha$ lantaq	12	✓	
40 $\mu$ ddH <sub>2</sub> O	480	✓	

cycling  
cond  
for  
broth  
except  
72°C 1.5 min  
in  $\alpha$ lantaq

95°C 5 min 1 cycle  
95°C 1 min  
59 1 min  
72 3 min 3 cycles  
95 1  
57 1  
72 3 3 cycles

95°C 1 min  
55°C 1 min  
72°C 3 min  
20 cycles

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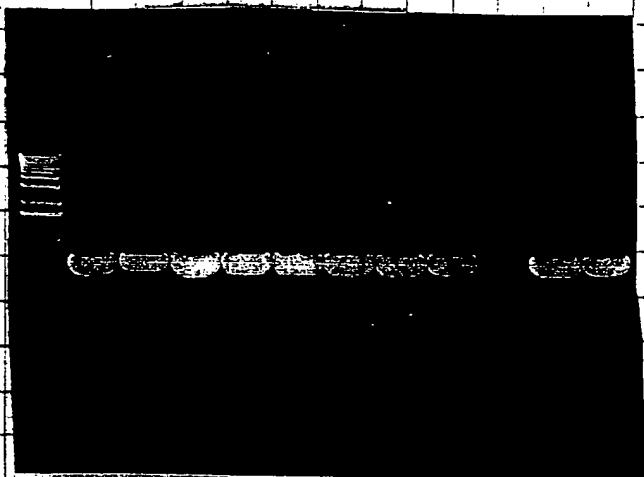
Invented by

Bethanne Zewel

Date

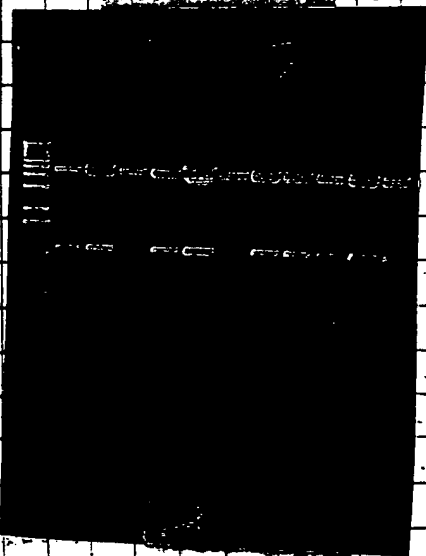
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49665 REN

Set up Xba digest for 49665



5x CDNA	12
2x React 2	24
1x Xba I	12
12x ddH <sub>2</sub> O	144

Set digest 1 hr 37°C

To Page No. \_\_\_\_\_

Witnessed &amp; Understood by me,

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Invented by

Bethanne Zewel

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